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Beta-1,3-glucan effect on sow antibody production and passive immunisation of progeny

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β -glucans are glucose homopolymers known to modulate immunity. Here, the β -glucan effect on sow antibody production and passive immunisation of neonatal pigs was analysed. Treatments included: (1) corn-soy fed Control group; (2) β -glucan; (3) *Actinobacillus pleuropneumoniae* (*App*) vaccination; and (4) β -glucan + *App* vaccination. Birth and weaning weights were not affected ($P > 0.05$) by treatment. Independent of treatment, IgA, IgG and IgM in colostrum were elevated and declined rapidly. Vaccination against *App* resulted in an increase ($P < 0.05$) in IgG and IgA specific for *App* serotypes 1, 5 and 7 in colostrum and milk, and a corresponding increase ($P < 0.05$) in serum in pigs at 4 d of age. However, β -glucan did not enhance specific *App* antibodies in pig serum. While the dosage used here did not enhance passive immunisation in pigs, β -glucan can be potentially efficacious as an oral adjuvant to enhance immunoglobulin production in response to vaccination.

Keywords: β -glucan; pig; colostrum; passive immunisation

Introduction

In utero, the foetal pig does not experience antigenic challenge due to the epitheliochorial placenta type of the porcine species, which also prevents transplacental passage of immunoglobulin molecules (Brambell, 1970; Chappuis, 1998; Roitt, Brostoff, & Male, 1998; Straw, Roth, & Saif, 1989; Watson, 1980). As a result, the neonate is born immunologically naïve and the immune system remains immature during the first weeks of life (Schwager & Schulze, 1997). While antibodies are produced by the neonate, they do not reach effective concentrations until three to four weeks of age (Roitt et al., 1998). Therefore, the neonate depends on immunoglobulin acquisition postnatally via colostrum ingestion and subsequent absorption across the intestinal epithelium (Schanbacher, Talhouk, & Murray, 1997). Colostrum is the source of immunoglobulins IgA, IgG and IgM that confer passive immunity to the neonate; moreover, it contains viable leukocytes capable of

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expressing cell-mediated immunity (Goldman, Cheda, & Garofalo, 1998; Hanson et al., 1984; Salmon, 1999). The permeability of the intestine to protein molecules is particularly high during the first 24–48 h after birth, making this time critical for survival and long-term growth of the neonate (Murata & Namioka, 1977). Additionally, trypsin inhibitors present in the colostrum and reduced intestinal proteolytic activity during this sensitive period facilitate the absorption of intact immunoglobulins (Chappuis, 1998; Kiriya, 1992; Murata & Namioka, 1977). Therefore, early pig viability is critically dependent on the passive transfer of maternal immunoglobulins and immunologic factors in colostrum that protect against pathogens until their own immune system matures.

Previous observations indicate that vaccination-induced immunoglobulin production can be increased in young pigs with oral administration of a highly purified β -glucan. Beta-1,3-glucans are complex glucose homopolymers, extracted and purified from the cell wall of yeast (Kim, Song, Lee, Cho, & Roh, 2006; Williams, Mueller, & Browder, 1996), that are reported to have broad anti-infective properties without inducing leukocyte activation or stimulation of pro-inflammatory cytokines (Antje et al., 1995; Cisneros, Gibson III, & Tzianabos, 1996; Onderdonk, Cisneros, Hinkson, & Ostroff, 1992). Moreover, immunoglobulin production is enhanced in response to vaccination in young pigs treated orally with highly purified β -glucan. Therefore, the study conducted herein was to determine the effect of β -glucan, as an oral adjuvant, on immunoglobulin production in late gestating and early lactating sows as a means to increase colostrum immunoglobulin concentration and subsequently enhancing the passive immunisation in the neonate (Michalek et al., 1998).

Materials and methods

Experimental design

To test the effect of β -glucan on maternal immunoglobulin production and subsequent passive immunisation of neonatal pigs, 24 crossbred sows (approximate age 3 years, parity 5.27 ± 0.67), three farrowing groups, $n = 9, 8$ and 7 sows/farrowing group) from the Texas A&M University-Kingsville Swine Center were utilised. Sows were fed a corn-soy (CS) based diet and maintained as per current industry practices. Five weeks prior to the farrowing dates, sows were assigned, by parity, to one of four treatment groups including: (1) CS fed Control group ($n = 6$); (2) β -glucan (5 mg/kg body weight; $n = 5$); (3) *Actinobacillus pleuropneumoniae* (*App*; Pneu-Pac™; Schering-Plough) vaccination (5 and 2 weeks pre-farrowing; $n = 6$); and (4) β -glucan (5 mg/kg body weight) + *App* vaccination (5 and 2 weeks pre-farrowing; $n = 7$). Sows in Treatment groups 2 and 4 received β -glucan as a feed additive until 2 weeks post-farrowing. Vaccination against *App* was used as a sentinel for antibody transfer (Baarsch et al., 1995; Haesebrouck, Chiers, Van Overbeke, & Ducatelle, 1997). Sows were moved to farrowing crates one week prior to their respective farrowing date. All sows farrowed at night or in the early morning; hence, the morning following farrowing was designated as d 0.5. Pigs within each litter were weighed and tattooed for permanent identification and limited cross-fostering within treatments ensured 7–11 piglets per litter. Milk samples were obtained from the sow on d 0.5, 4, 8, 16 and on the day of weaning. Samples were collected from the anterior region of the udder following the removal of the pigs for 45 min and administration of oxytocin (20 USP),

a stimulator of mammary myoepithelial cell contraction. Samples were stored at -80°C until analysis for immunoglobulins IgG, IgA and IgM. Blood samples were collected via jugular veinpuncture from each pig on the d 4, 8, 16 and on day of weaning. Serum was harvested and stored at -80°C until analysis for immunoglobulins IgG, IgA and IgM.

Serum and milk analysis

Serum and milk concentrations of immunoglobulins were determined using a double antibody sandwich enzyme link immunosorbent assay (ELISA) specific for porcine immunoglobulins as described by Leiner, Franz, Strutzberg, and Gerlarch (1999) and Nemec, Butler, Hidioglou, Farnworth, and Nielsen (1994). Immunoplates (Nunc Maxisorp, 439454) were incubated overnight with affinity purified goat anti-pig IgG, IgM or IgA (100 μl /well; Bethyl Laboratories, Montgomery, TX) in 0.1 M NaCO_3 (pH 8.2). Plates were washed three times with wash buffer (phosphate buffered saline; PBS+0.05% Tween-20). Blocking buffer (PBS+1% bovine serum albumin) was added (200 μl /well), to block non-specific binding, and incubated for 30 min. Plates were washed three times with wash buffer and serum samples, diluted in PBS+Tween-20 (0.05%)+BSA (1%), were added to the plates and incubated for 2 h at room temperature. Following three washes with wash buffer, affinity purified goat anti-pig IgG, IgM or IgA-Fc conjugated with horseradish peroxidase (1/2000) was added (100 μl /well) and incubated for 1 h at room temperature and then washed four times. Enzyme substrate buffer ([2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS))+0.0015% H_2O_2 , pH 4.5) was added (100 μl /well) and incubated for 1 h at room temperature. Optical densities were determined at 405 nm (EL 311; Bio-Tek Instruments Inc., Winooski, VT). Sample IgG, IgM and IgA concentrations were determined by comparison to standard curves generated with purified swine immunoglobulins (Bethyl Laboratories, Montgomery, TX).

Statistical analyses

Milk and serum samples were analysed using the General Linear Model (GLM) procedure of SAS (1985). The partitioned sources of variation included treatment, farrowing group, parity and their respective interactions. Specific treatment comparisons were made using non-orthogonal contrasts. Comparisons included: Control (1) versus *App* vaccine (3) = V; Control (1) versus β -glucan (2) = B; and *App* vaccine (3) versus β -glucan + *App* vaccine (4) = BV.

Results

Pig production values were not altered ($P > 0.05$) by β -glucan as indicated by similar number born alive, birth weights and weaning weights (9.71 ± 0.49 , 1.29 ± 0.04 and 5.47 ± 0.15 kg, respectively) among the four treatment groups. Milk content of IgA, IgG and IgM were initially high but declined rapidly throughout lactation (Table 1). Immunoglobulin G was higher than IgA and IgM in colostrum on d 0.5 having a mean value of 43.76 ± 4 mg/ml; however, by d 4 of lactation, it decreased to 3.1% of its initial concentration. Immunoglobulin G continued to decrease ($P < 0.05$)

Table 1. Effect of oral β -glucan fed to gestating sows on milk IgA, IgG and IgM production during d 0.5 to day of weaning.

	Treatment ^a					
Time, day	1	2	3	4	SEM ^b	Comparison ^c
IgA, mg/ml						
0.5	6.99	7.79	7.77	7.04	±0.72	V, BV
4	5.10	4.80	4.81	5.01	±0.44	
8	3.64	3.66	5.14	3.37	±0.23	
16	3.21	3.60	3.45	3.22	±0.25	
Weaning	3.04	3.46	4.95	3.93	±0.30	
IgG, mg/ml						
0.5	51.91	46.71	37.14	39.28	±4.26	
4	1.62	1.19	1.14	1.43	±0.15	
8	0.49	0.39	0.48	0.70	±0.06	
16	0.29	0.30	0.32	0.41	±0.03	
Weaning	0.26	0.43	0.34	0.42	±0.04	
IgM, mg/ml						
0.5	3.48	3.54	2.14	2.91	±0.18	V
4	2.54	2.26	1.82	2.25	±0.12	V
8	1.50	1.18	0.90	1.54	±0.08	V, BV
16	1.08	0.91	0.72	1.09	±0.05	V, BV
Weaning	1.21	1.16	0.84	1.44	±0.09	BV

^aSows were assigned to one of four treatments: (1) no *App* vaccine, 0 mg/kg β -glucan; (2) no *App* vaccine, 5 mg/kg β -glucan; (3) *App* vaccine at 5 and 2 weeks prior to farrowing, 0 mg/kg β -glucan; and (4) *App* vaccine at 5 and 2 weeks prior to farrowing, 5 mg/kg β -glucan. ^bStandard error of the least square mean.

^cSignificant ($P < 0.05$) treatment effect comparisons within day are marked: Treatment 1 versus Treatment 3 (\pm vaccine; V); Treatment 1 versus Treatment 2 (\pm β -glucan; B); and Treatment 3 versus Treatment 4 (\pm vaccine \pm β -glucan; BV).

reaching nadir concentrations on d 8 of lactation. The total concentration of IgG and rate of decline were not affected by treatment.

In colostrum, the initial IgA mean value was 7.4 ± 0.72 mg/ml and began decreasing on d 4; however, by d 8 concentrations remained steady throughout the lactation period. Treatment did not affect total concentrations of IgA in milk or serum. By d 8 of lactation, a suppression of IgA occurred in the *App* vaccinated sows receiving β -glucan (Treatment 4; Table 2). However, this was the only time that this phenomenon appeared to have occurred. Immunoglobulin IgA was the predominant antibody in the milk throughout lactation and was higher ($P < 0.05$) in colostrum than in the serum of pigs.

Immunoglobulin IgM concentrations were the lowest in colostrum with an initial mean value of 3.02 ± 0.18 mg/ml (Table 1). *App* vaccination resulted in a slight ($P = 0.1$) suppression in colostral concentrations of IgM (Treatment 3), which was maintained throughout lactation. In contrast, β -glucan appeared to overcome ($P < 0.05$) the suppression on IgM content (Treatment 4) on d 8, 16 and at weaning (Table 1). It is not clear how β -glucan reversed the suppression on IgM following *App* vaccination. Serum IgG was higher ($P < 0.05$) than IgA and IgM in all pigs until weaning; reaching peak concentrations on d 4 and declining until weaning (Table 2).

Table 2. Effect of oral β -glucan fed to gestating sows on pig's serum IgA, IgG and IgM from d 0.5 to day of weaning.

	Treatment ^a					
Time, day	1	2	3	4	SEM ^b	Comparison ^c
IgA, mg/ml						
4	2.85	2.87	4.88	3.13	±0.25	V, BV
8	0.59	1.15	1.59	0.91	±0.11	V, BV
16	0.13	0.15	0.19	0.15	±0.01	
Weaning	0.15	0.15	0.16	0.14	±0.01	
IgG, mg/ml						
4	31.58	32.04	30.55	30.23	±1.28	
8	22.49	28.44	25.92	25.60	±1.02	
16	13.21	13.28	17.36	14.50	±0.92	
Weaning	8.82	8.13	10.20	7.95	±0.50	
IgM, mg/ml						
4	1.59	1.99	1.60	1.74	±0.14	
8	0.70	0.94	0.78	0.98	±0.08	
16	0.58	0.60	0.59	0.69	±0.05	
Weaning	0.79	0.64	0.70	0.78	±0.08	

^aSows were assigned to one of four treatments: (1) no *App* vaccine, 0 mg/kg β -glucan; (2) no *App* vaccine, 5 mg/kg β -glucan; (3) *App* vaccine at 5 and 2 weeks prior to farrowing, 0 mg/kg β -glucan; and (4) *App* vaccine at 5 and 2 weeks prior to farrowing, 5 mg/kg β -glucan. ^bStandard error of the least square mean.

^cSignificant ($P < 0.05$) treatment effect comparisons within day are marked: Treatment 1 versus Treatment 3 (\pm vaccine; V); Treatment 1 versus Treatment 2 (\pm β -glucan; B); and Treatment 3 versus Treatment 4 (+ vaccine \pm β -glucan; BV).

In contrast to concentrations of IgG in sows' milk, pig serum IgG did not drastically decrease. Neither *App* vaccination nor β -glucan affected total serum IgG concentrations. Unlike IgG, serum IgA decreased ($P < 0.05$; Table 2) compared to a moderate decline that occurred in sows' milk. In contrast to IgA concentrations in milk, serum IgA was higher ($P < 0.05$) on d 4 and 8 in the *App* vaccinated treatment groups, independent of β -glucan treatment. Treatments did not affect total serum concentrations or the rate of decline of IgA in milk or pig serum.

Serum concentrations of IgM were highest on d 4 and significantly ($P < 0.05$) declined until weaning (Table 2). Contrary to the concentration of IgM in milk in the *App* vaccinated sows, serum IgM was not suppressed, which is a difference that is not presently clear. Treatments did not affect serum concentrations or the rate of decline of IgM in milk or pig serum.

Vaccination against *App* (Treatments 3 and 4) resulted in an increase ($P < 0.05$) in IgG and IgA specific for *App* serotypes 1, 5 and 7 in colostrum and milk (Table 3). This corresponded to an increase ($P < 0.05$) in pig serum on d 4 (Table 4).

Discussion

In contrast to Dritz et al. (1995), pig performance was not altered ($P < 0.05$) by β -glucan as indicated by similar birth weights and weaning weights among the four

Table 3. Influence of oral β -glucan fed to gestating sows on *Actinobacillus pleuropneumoniae* (App) IgG, serotypes 1, 5 and 7 on pig serum at 4 d of age.

Serotypes	Treatment ^a				SEM ^b	Comparison ^c
	1	2	3	4		
1	0.124	0.080	0.423	0.404	0.028	V
5	0.363	0.261	0.584	0.608	0.034	V
7	0.359	0.249	0.543	0.579	0.038	V

^aSows were assigned to one of four treatments: (1) no App vaccine, 0 mg/kg β -glucan; (2) no App vaccine, 5 mg/kg β -glucan; (3) App vaccine at 5 and 2 weeks prior to farrowing, 0 mg/kg β -glucan; and (4) App vaccine at 5 and 2 weeks prior to farrowing, 5 mg/kg β -glucan. ^bStandard error of the least square mean.

^cSignificant ($P < 0.05$) treatment effect comparisons within day are marked: Treatment 1 versus Treatment 3 (\pm vaccine; V), Treatment 1 versus Treatment 2 (\pm β -glucan; B), and Treatment 3 versus Treatment 4 (+ vaccine \pm β -glucan; BV).

treatment groups. However, this is likely attributed to the administration of β -glucan directly to the sow rather than the pig, which diminished potentially unfavourable effects on pig's performance. IgA, IgG and IgM content in milk were initially high but declined rapidly throughout lactation. Immunoglobulin G was higher than IgA and IgM in colostrum on d 0.5, however, by d 4 of lactation, it decreased to 3.1% of its initial concentration. This is similar to a study by Frenyo, Pethes, Antal, and Szabo (1981) that reported a decrease in milk IgG to 3.2% of the initial concentration by d 5 of lactation. The total concentration of IgG and rate of decline were not affected by treatment. The physiological process of providing large amounts of IgG in colostrum might be an adaptation that compensates the pig's inability to obtain antibodies prior to birth and to provide the pig with protection against systemic infectious pathogens in the first days of life. Likewise, the quick decline in IgG content coincides with the subsequent shut-off of immunoglobulin absorption in the gut of the neonate (Butler, 1979).

In colostrum, IgA appeared to begin decreasing on d 4; however, by d 8, concentrations remained steady throughout the lactation period. Treatment did not affect total concentrations of IgA in milk or serum. By d 8 of lactation, a suppression

Table 4. Influence of oral β -glucan fed to gestating sows on *Actinobacillus pleuropneumoniae* (App) IgG, serotypes 1, 5 and 7 on pig serum at 4 d of age.

Serotypes	Treatment ^a				SEM ^b	Comparison ^c
	1	2	3	4		
1	0.016	0.006	0.194	0.145	0.005	V, BV
5	0.077	0.066	0.439	0.337	0.010	V, BV
7	0.123	0.136	0.462	0.376	0.009	V, BV

^aSows were assigned to one of four treatments: (1) no App vaccine, 0 mg/kg β -glucan; (2) no App vaccine, 5 mg/kg β -glucan; (3) App vaccine at 5 and 2 weeks prior to farrowing, 0 mg/kg β -glucan; and (4) App vaccine at 5 and 2 weeks prior to farrowing, 5 mg/kg β -glucan. ^bStandard error of the least square mean.

^cSignificant ($P < 0.05$) treatment effect comparisons within day are marked: Treatment 1 versus Treatment 3 (\pm vaccine; V); Treatment 1 versus Treatment 2 (\pm β -glucan; B); and Treatment 3 versus Treatment 4 (+ vaccine \pm β -glucan; BV).

of IgA occurred in the *App* vaccinated sows receiving β -glucan (Treatment 4; Table 2). However, this was the only time that this phenomenon appeared to have occurred; therefore, it does not likely represent an effect of either *App* vaccination or β -glucan. Immunoglobulin IgA was the predominant antibody in the milk throughout lactation, which is supported by numerous studies (Butler, 1979; Porter, 1973; Salmon, 1999; Watson, 1980). Moreover, IgA was higher ($P < 0.05$) in colostrum than in the serum of pigs, which is similar to a previous report by Bourne and Curtis (1973). A consistent level of IgA in milk throughout lactation is an important component in mucosal immunity that ensures the pig with enteric and mucosal protection. Immunoglobulin IgA is expressed in the mucosa as a secretory IgA and instead of being absorbed through the intestinal tract it lines the mucosa and prevents mucosal infectious agents from adhering to the epithelium (Porter, 1973).

Immunoglobulin IgM concentrations were the lowest in colostrums and *App* vaccination resulted in a slight suppression in colostrum concentrations of IgM (Treatment 3), which was maintained throughout lactation. In contrast, β -glucan appeared to overcome the suppression on IgM content (Treatment 4) on d 8, d 16 and at weaning. It is not clear how β -glucan reversed the suppression on IgM following *App* vaccination. Similar to a report by Gomez, Phillips, and Goforth (1998), serum IgG was higher ($P < 0.05$) than IgA and IgM in all pigs until weaning; reaching peak concentrations on d 4 and declining until weaning (Table 2).

In contrast to concentrations of IgG in sows' milk, pig serum IgG did not drastically decrease. Neither *App* vaccination nor β -glucan affected total serum IgG concentrations. Unlike IgG, serum IgA decreased compared to a moderate decline that occurred in sows' milk. Serum concentrations of IgA are typically low as they reside primarily in and around mucosal epithelia. In contrast to IgA concentrations in milk, serum IgA was higher on d 4 and d 8 in the *App* vaccinated treatment groups, independent of β -glucan treatment. At present, the difference between milk IgA and pig serum IgA in the *App* vaccinated groups is not clear. Treatments did not affect total serum concentrations or the rate of decline of IgA in milk or pig serum.

Serum concentrations of IgM were highest on d 4 and significantly declined until weaning. Contrary to the concentration of IgM in milk in the *App* vaccinated sows, serum IgM was not suppressed, which is a difference that is not presently clear. Treatments did not affect serum concentrations or the rate of decline of IgM in milk or pig serum.

Vaccination against *App* (Treatments 3 and 4) resulted in an increase in IgG and IgA specific for *App* serotypes 1, 5 and 7 in colostrum and milk, and corresponded to an increase in pig serum on d 4. Therefore, β -glucan can be efficacious as an oral adjuvant to enhance immunoglobulin production in response to vaccination. However, at the dosage used in the current study, β -glucan was not effective at enhancing passive immunisation in pigs.

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